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Size-dependent effects of temperature and food stress on energy reserves and starvation resistance in yellow dung flies

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ABSTRACT

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Organism: Yellow dung flies, *Scathophaga stercoraria* (Diptera: Scathophagidae), artificially selected for large and small body size in the laboratory for 11 (24) generations.

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Conclusions: Limited larval food and high temperature decreased life span. Life span increased markedly with body size due to the greater energy content of larger flies. Small selection line flies had relatively more energy and longer life spans under complete starvation, indicating a compensatory correlated genetic response to body size selection. The larger males suffered most under multiple stresses. Our results support the relative efficiency hypothesis, which more than compensates for the greater absolute energy demand of larger individuals.

Keywords: allometry, artificial selection, body size, condition, food limitation, life history, metabolic rate, reproduction, starvation resistance, survival, thermal stress, viability.

INTRODUCTION

Large body size appears to be generally favoured by selection in most organisms. Many advantages of large size are documented, whereas evidence for putative disadvantages is scarce in comparison (Blanckenhorn, 2000). Sexual selection in males (Andersson, 1994) and fecundity selection in females (Shine, 1988; Honek, 1993) are the main forces selecting for large size. Even viability is often positively correlated with body size (reviewed in Roff, 1992, p. 117; Andersson, 1994; e.g. Partridge and Farquhar, 1983). Nevertheless, viability selection at the juvenile or adult life stages is believed to be the main factor balancing evolution towards ever larger body sizes

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(Schluter *et al.*, 1991; Blanckenhorn, 2000, 2005). Viability disadvantages can be caused by reduced agility (Ghiselin, 1974; Neems *et al.*, 1990; Andersson, 1994) and higher detectability of large-sized individuals so that they become preferred objects of predators or parasites (Werner, 1986; Clancy and Price, 1987; Loader and Damman, 1991; Spitze, 1991; Fincke *et al.*, 1997). Additionally, sustaining larger sizes can increase energetic costs, especially under resource limitation (Partridge and Fowler, 1993; Santos *et al.*, 1994; Blanckenhorn *et al.*, 1995; Wikelski *et al.*, 1997). However, detailed studies of these potential physiological costs of large size and their role in limiting body size evolution are scant.

One reason why the life-history costs of large body size may be hard to detect in nature is that presumed trade-offs underlying the evolution of adaptive life histories often become visible only in stressful environments. This is because when resources are not limited, individuals can invest maximally in all traits. Therefore, a good environment, or good condition, often masks expected life-history costs and trade-offs (Ojanen *et al.*, 1979; van Noordwijk and de Jong, 1986; Alatalo *et al.*, 1990; Schluter *et al.*, 1991; Rowe and Houle, 1996; Talloen *et al.*, 2004). This means that it is imperative to experimentally investigate individuals under stressful conditions. A second reason is that in many species large individuals may be produced but die early during development. In any study of selection on adult body size, these individuals cannot be measured and therefore would not be part of the size distribution (Blanckenhorn, 2000). A third reason why disadvantages of large size might not become apparent is that the function linking fitness to body size may be largely flat so that (steep) fitness decrements may only occur at the fringes of the naturally existing size distribution. One way of addressing the last two problems is to artificially select for larger than natural organisms in the laboratory and assess whether intrinsic or extrinsic costs of large body size would become apparent even though in nature they are not. This was done here.

In the physiological literature, there are two contrasting hypotheses concerning the energetic costs of animals in relation to body size. The more intuitive hypothesis, here termed the absolute energy demand hypothesis, is based on the assumption that larger individuals have an absolutely higher energy requirement for maintaining their body functions. Thus energy reserves, and consequently food consumption and/or assimilation efficiency, must increase (isometrically) with body size (Calder, 1984; Lindstet and Boyce, 1985; Blanckenhorn *et al.*, 1995; Donohue *et al.*, 2002), and large animals are predicted to do worse in limited environments. For example, Wikelski *et al.* (1997) showed that under extreme food limitation, the higher energy demand of large iguanas led to higher mortality. In contrast, large body size apparently confers more efficient energy use because mass-specific metabolic rate decreases with body size [Kleiber's (1932) 3/4-power law (Hemmingsen, 1960; Heusner, 1982; West *et al.*, 1997; Dodds *et al.*, 2001; but see Kozlowski and Konarzewski, 2004)], here termed the relative efficiency hypothesis. This predicts lower mortality of larger animals because their accumulated energy should last longer, potentially even in limited environments. While Kleiber's law originally referred to global comparisons among species with vastly different body sizes (i.e. the macro-evolutionary level), in principle it should apply equally at the micro-evolutionary level within species (e.g. Nakaya *et al.*, 2005). These two hypotheses are, of course, not mutually exclusive, as fitness ultimately depends on their cumulative, net effects.

Here, we specifically address the energetic costs of large body size under food and temperature stress. We use yellow dung flies, *Scathophaga stercoraria* (Diptera: Scathophagidae), a species associated with cattle dung that is found in north-temperate regions of the Old and the New World. Yellow dung flies feed on dung as larvae, and require nectar for energy and prey to reproduce (Foster, 1967). They are adapted to cold temperatures, and in the southern regions of their distribution such as Britain or Switzerland they typically

disappear from the pastures, where they reproduce, during the hot summer months, not returning until early autumn when temperatures decrease again (Parker, 1970; Gibbons, 1987; Blanckenhorn *et al.*, 2001). The body size of yellow dung flies is highly phenotypically plastic: both food limitation and higher temperatures during the pre-adult stage result in a decrease in adult size (Amano, 1983; Blanckenhorn, 1997, 1998). We worked with yellow dung flies artificially selected for large and small body size in the laboratory. We measured the energy content of teneral (i.e. freshly eclosed) adult flies as well as their (physiological) adult life span under complete starvation (in different individuals), referred to as starvation resistance in the *Drosophila* literature (e.g. Harshman *et al.*, 1999; Robinson *et al.*, 2000; Hoffmann *et al.*, 2001). The brief period during and after adult eclosion is crucial for many insects, as they then are very susceptible to predators and parasites; they also initially cannot feed (because they are still soft), and if environmental conditions further preclude feeding (e.g. due to extended rainfall), starvation during this period is a realistic threat and teneral energy may be crucial for survival. We performed our experiments at both low and high (i.e. stressful) temperatures and in unlimited and limited (i.e. stressful) food conditions during the pre-adult and adult stages. If effects of the absolute energy demand hypothesis take precedence, we anticipated unfavourable environmental conditions to be more stressful for large individuals, resulting in a lower energy content and consequently shorter life spans under starvation. Alternatively, if effects of the relative efficiency hypothesis take precedence, we anticipated that even under unfavourable conditions large-sized individuals can more than compensate for their absolutely higher energy demands by using energy more efficiently, thus resulting in more energy stored and higher starvation resistance.

MATERIALS AND METHODS

Selection lines

The purpose of our body size selection lines (detailed in Teuschl *et al.*, in press) was to artificially increase the natural body size range of yellow dung flies to examine presumed cryptic costs of large body size (cf. above). The selection lines were started from eggs laid in the laboratory by 120 mature female *Scathophaga stercoraria* randomly collected in April and May 2000 near Zürich, Switzerland. Twenty-five eggs from each clutch (i.e. family) were transferred into 50-ml plastic containers containing 80 g cow dung [more than 2 g of dung per larva can be considered unlimited larval food conditions (Amano, 1983)], and the offspring were raised at 20°C and 60% relative humidity. Fifty males and 50 females from as many families as possible were randomly assigned to one of two replicates each of a small (S), a large (L), and a control (C) body size line. Within each line, 50 pairs were formed randomly to start selection with the F2 generation. Every generation, the head widths (a practical surrogate of overall size) of 150 males (if possible three from each family) were measured, and the 50 smallest (S) or largest (L) or 50 randomly chosen (C) males were allowed to reproduce with 50 randomly chosen females of the same line. To produce the next generation, 20–25 eggs of each selected clutch were transferred into 50-ml plastic containers with 80 g dung kept at a constant 20°C and 60% relative humidity until offspring emerged after 19–22 days. Adult flies were kept singly in 100-ml vials until sexually mature at the same conditions with water, sugar, and *Drosophila melanogaster ad libitum* as prey. Selection was performed for a total of 24 generations, although most of our experiments here were performed with flies from generation 11. To offset possible

inbreeding effects, flies from the two replicates within each selection line were crossed and propagated without selection for one more generation before performing our experiments described below.

Starvation resistance experiment

Yellow dung flies used for this experiment stemmed from the crossed replicates of our selection lines at generation 11. Females of all three lines (small, control, large) were allowed to mate and lay eggs. Eggs were transferred into containers with limited (20 eggs per 5 g dung) or unlimited (15 eggs per 80 g dung) dung conditions, thus manipulating larval density and food availability simultaneously as dung is both food and environmental medium for the larvae (cf. Amano, 1983). For each dung treatment, half of the containers were held at 20°C and the other half at stressfully hot conditions (cf. Ward and Simmons, 1990) of 25°C (no split brood design). From day 16 onwards, all containers were checked three times a day for emerging flies, and emergence (i.e. egg-to-adult development time) and head width were noted. Thereafter, all flies were kept singly in 50-ml glass vials with moistened cotton but without food. Flies from both pre-adult treatments were randomly assigned to one of two adult temperatures in a crossed design: half of the emerging flies from each larval environment were kept at 20°C and the other half at 25°C, 60% relative humidity, and a 12-h photoperiod. All flies were checked for death three times a day (morning, midday, evening).

Teneral energy content of line flies in different dung conditions

For teneral energy content of line flies, we produced two different data sets. One data set was obtained using line flies from both replicates of each selection line at generation 11. The other energy data set was collected at the end of selection using generation 24, after replicates had been crossed to avoid inbreeding effects. As above, larvae were allowed to develop in abundant and limited dung conditions. All containers were held at 20°C. From day 16 onwards, dung containers were checked daily for emerging flies. Emerged flies were only used for energy analysis if no dead fly was present in the container to avoid measuring flies that might have already fed on their siblings (adult flies are predatory). Teneral flies were immediately transferred into a test tube filled with 100% ethanol, which was heated for 5 min at 90°C and thus fixated for later analysis as described below.

Teneral energy content of field-derived flies at different temperatures and dung conditions

To assess if or to what extent artificial selection on body size might have led to genetically correlated changes in energy content, the body size and energy content of field-derived flies were additionally measured to obtain a baseline for the phenotypic relationship between body size and energy content that could be compared with the line flies. In June 2002, we collected about 30 copulating *S. stercoraria* females from the field and let them lay eggs in the lab. To minimize carry-over maternal effects, the next generation was reared under standard laboratory conditions (as above) and mated randomly. Eggs were transferred to limited and unlimited dung conditions and larvae were again reared at normal (20°C)

and hot (25°C) temperatures. Dung containers were checked twice a day for emerging flies, and flies were immediately fixated in 100% ethanol and heated for later energy analyses as above.

Energy analysis

The flies' energy content was analysed by the photometric method of Van Handel (1985a, 1985b), enabling us to measure the glycogen, glucose, and lipid contents of an individual at the same time. Glycogen and lipids are the main long-term energy storage compounds of most organisms. Lipids and carbohydrates were extracted using a chloroform/methanol mixture. Interfering chitin was dissolved by potassium hydroxide (KOH). Carbohydrates were subsequently quantified photometrically after an anthrone–sulphuric acid reaction (Van Handel, 1985a), and lipids after a vanillin–phosphoric acid reaction (Van Handel, 1985b).

Statistical methods

Because the replicate selection lines did not differ (see Results), starvation resistance was analysed using analysis of covariance, with replicate line as a blocking factor (as opposed to the nested model described below for energy content), sex, pre-adult temperature, adult temperature, dung treatment, and selection line as crossed fixed factors, and phenotypic body size (head width) as a covariate. This model takes into account the fact that selection line and dung treatment, although both highly correlated with body size, potentially have additional size-independent effects.

Lipid, glycogen, and glucose content were analysed separately in two steps. To first test for systematic correlated genetic responses to the selection regime, the three energy components of teneral selection line flies were analysed as nested analyses of covariance with replicate line nested in the line × sex × environmental treatment combination, and phenotypic body size as a covariate. The logic here is that if selection line replicates (randomly) diverge genetically, this is likely to equally affect selection line (i.e. genotype) × environment interactions. In a second step, teneral flies of the 24th generation (after crossing both replicates) were analysed using analogous (i.e. phenotypic) analyses of covariance to investigate potential further evolution in the laboratory of physiological responses in the selection lines. The field-derived flies were analysed analogously using analyses of covariance with dung, pre-adult temperature treatment, and sex as crossed factors and body size as the covariate. In general, all results of all our analyses consequently are size-corrected (as body size was a covariate). Also, non-significant higher-order interactions were dropped from the final models.

A number of data points were obvious measurement or methodological errors (flies apparently having had inadvertent access to some food, photometric errors, etc.). To legitimately delete these extremes, we in practice excluded all data points that were more than 3.5 standard deviations away from the mean (life span data: 4%; energy data: 2%). In all statistical analyses, the different energy components (glucose, glycogen, and lipid), which represent volumes, were cube-root-transformed to linearize and thus keep them at the same biological scale as our body size measure head width (which was consequently not transformed). Life span was square-root transformed (equivalent to a log-transformation) for statistical reasons.

RESULTS

Body size distributions

Artificial body size selection resulted in significantly divergent body size distributions of our three selection lines at generation 11 (both replicates combined), large line flies being about 10% larger than any fly in nature in our population: large males and females: 2.73 ± 0.26 (95% CI) and 2.44 ± 0.32; control males and females: 2.52 ± 0.19 and 2.22 ± 0.18; small males and females: 2.34 ± 0.18 and 2.05 ± 0.18 (all at unlimited dung and 20°C; $n > 50$); field-derived males and females: 2.56 ± 0.17 and 2.18 ± 0.14 ($n > 30$).

Starvation resistance experiment

In general, life span without food (i.e. starvation resistance) strongly increased with body size, similarly for the sexes (body size effect in Table 1; Fig. 1). Since the two replicates plus the replicate cross overall did not differ, we tested all effects against the global (phenotypic)

Table 1. Results of analysis of variance of (square-root transformed) starvation resistance of teneral yellow dung flies with pre-adult temperature, dung treatment, adult temperature, sex and line as fixed factors, body size as a covariate, and replicate as a blocking factor (because it was not significant; all non-significant higher-order interactions were dropped from the model)

	d.f.	MS	*F*	*P*
Intercept	1	27.0804	54.359	<0.001
Sex	1	13.256	25.915	**<0.001**
Adult temperature	1	431.522	843.656	**<0.001**
Dung	1	19.516	38.155	**<0.001**
Pre-adult temperature	1	0.201	0.393	0.531
Line	2	8.724	17.055	**<0.001**
Replicates	2	0.761	1.489	0.212
Body size	1	41.757	81.637	**<0.001**
Sex × adult temperature	1	2.972	5.809	**0.016**
Sex × dung	1	0.394	0.771	0.380
Sex × pre-adult temperature	1	5.855	11.446	**0.001**
Sex × line	2	0.332	0.650	0.522
Adult temperature × dung	1	10.673	20.867	**<0.001**
Adult temperature × pre-adult temperature	1	2.765	5.405	**0.020**
Adult temperature × line	2	0.716	1.400	0.247
Dung × pre-adult temperature	1	2.786	5.446	**0.020**
Dung × line	2	0.477	0.933	0.394
Pre-adult temperature × line	2	0.745	1.457	0.233
Sex × adult temperature × dung	1	1.174	2.296	0.130
Sex × adult temperature × pre-adult temperature	1	0.455	0.890	0.346
Sex × adult temperature × line	2	2.376	4.646	**0.010**
Sex × dung × pre-adult temperature	1	1.774	3.469	0.063
Sex × dung × line	2	0.696	1.360	0.257
Sex × pre-adult temperature × line	2	0.203	0.397	0.672
Sex × adult temperature × dung × pre-adult temperature	2	2.039	3.986	**0.019**
Error	802	0.511		

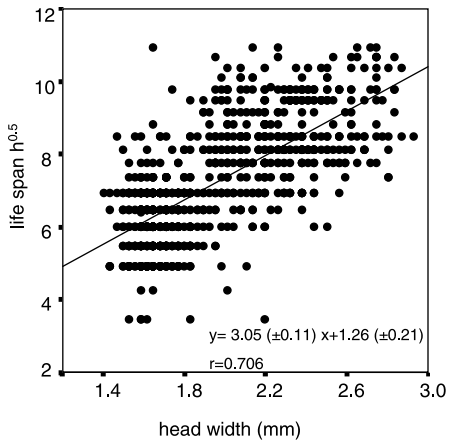

Fig. 1. Relationship of life span under complete starvation (starvation resistance) of teneral yellow dung flies from all selection lines and both sexes combined with body size (generation 11). The regression equation (± standard error) and the correlation are also given.

error (Table 1). Comparing selection lines, small line flies lived significantly longer than large line flies (contrast: $t_{533} = 4.81$, $P < 0.001$), while large line flies did not differ from the controls when correcting for body size differences (contrast: $t_{517} = 0.54$, $P = 0.625$; line effect in Table 1; Fig. 2). As anticipated, unfavourable environmental conditions such as a high temperature during the adult stage and limited dung during the pre-adult stage shortened life span markedly (dung and adult temperature effects in Table 1; Fig. 3), with the reduction being most pronounced at unlimited dung and hot temperature (adult temperature × dung effect in Table 1; Fig. 3). When correcting for the body size difference, females survived longer than males (sex effect in Table 1; Figs. 2 and 3). In the control line, this difference in life span between the sexes was much greater at high adult temperature, leading to a significant sex × adult temperature × line interaction (Table 1, Fig. 2). Moreover, all environmental factors and sex together influenced starvation resistance, resulting in a complex four-way interaction (Table 1). In general, males suffered disproportionately more under stressful conditions (Fig. 3).

Teneral energy content of line flies at different dung conditions

Generally, the energy content of teneral line flies (of generation 11) consisted mainly of lipids and was positively related to body size (Fig. 4). Nested analysis of covariance revealed that artificial body size selection produced significantly divergent responses in energy content in our replicate lines, for reasons that are unknown (Table 2). While glucose and glycogen content were only affected by body size, lipid content was additionally influenced by the other factors (Table 2). Males accumulated significantly more lipids during the pre-adult stage than females, primarily under unlimited dung conditions (sex effect and sex × dung interaction in Table 2; Fig. 5). Furthermore, lipid content was generally greater under unlimited dung conditions (dung effect in Table 2), which was most pronounced in the large and small but not in the control lines, leading to a significant line × dung interaction (Table 2, Fig. 5). Lipid content overall increased from large to small line flies, although at limited dung only (line × dung effect in Table 2; Fig. 5), indicating that there was

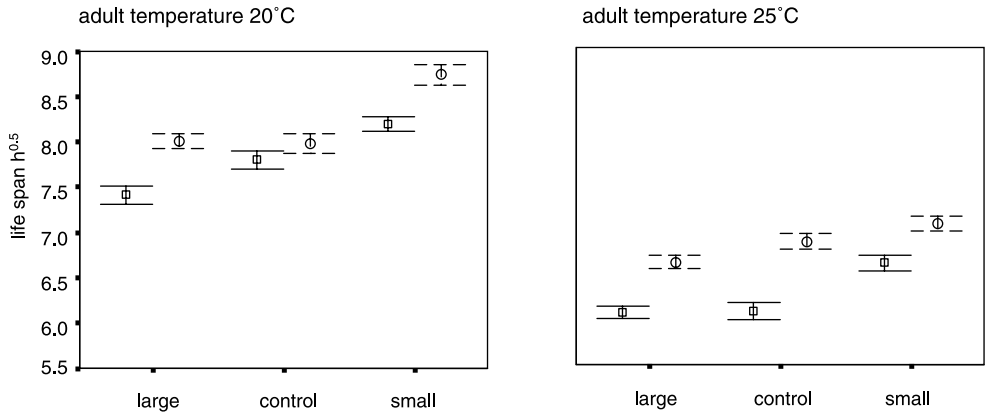

Fig. 2. Size-corrected mean (± standard error) life span under complete starvation (starvation resistance) of teneral yellow dung flies from three body size selection lines at two adult holding temperatures, separated by sex (generation 11; all replicates combined). □, male; ○, female.

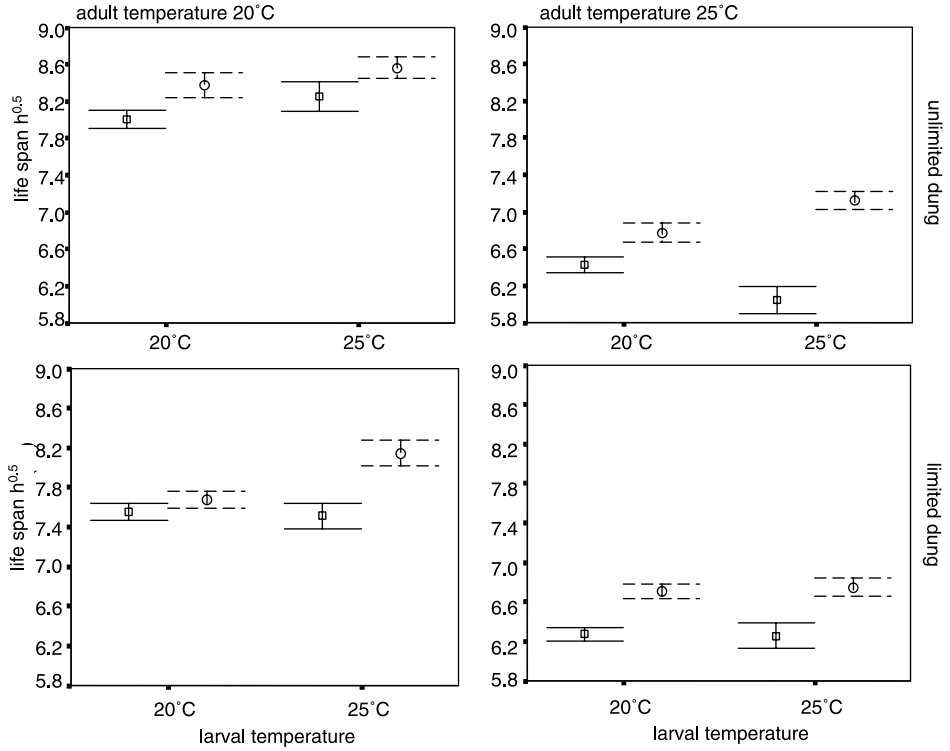

Fig. 3. Size-corrected mean (± standard error) life span under complete starvation (starvation resistance) of teneral yellow dung flies from all selection lines combined at different pre-adult temperatures and unlimited (top) and limited dung conditions (bottom), at both low (left) and high adult temperatures (right; generation 11). □, male; ○, female.

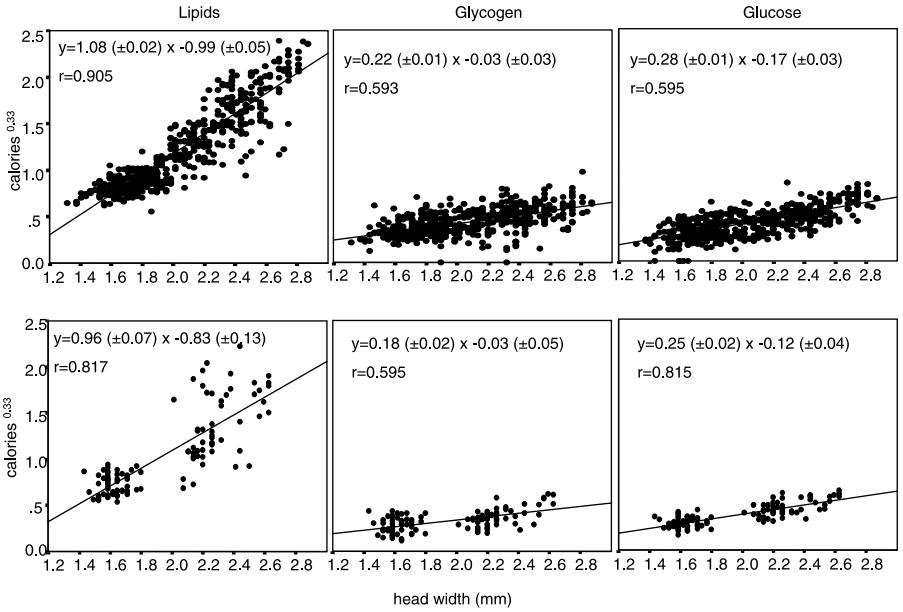

Fig. 4. Individual energy content of teneral body size selection line flies of generation 11 (top) and field-derived yellow dung flies (bottom) as a function of body size. The regression equation (± standard error) and the correlation are also given.

some correlated response of lipid storage to selection on body size. Note, however, that the replicates differed (replicate effects in Table 2), indicating some degree of divergent (laboratory) evolution in terms of energetics, for reasons we are unaware of.

To verify whether these effects remained or even strengthened as selection proceeded, the crossed replicates (one per line) of the 24th generation were analysed using analogous factorial analysis of covariance. In general, the differences between the lines magnified: by generation 24, small line flies had accumulated more energy than large line flies for all three energy components (line and line × sex interactions for all three energy components in Table 2; Figs. 5 and 6). However, while in the 11th generation males contained more lipids under unlimited dung conditions than females, this effect reversed over generations: now size-matched females stored relatively more lipids (sex × dung interactions in Table 2; Figs. 5 and 6). Furthermore, by generation 24, line flies had developed relatively greater teneral lipid and glycogen contents at limited than at unlimited dung (dung effect and line × dung interaction in Table 2; Figs. 5 and 6), whereas in the 11th generation the converse was the case. This resulted in a three-way line × dung × sex interaction for lipids, as the pattern is only marginally visible in females but very distinct in males (Table 2; Fig. 5).

Teneral energy content of field-derived flies under different dung and temperature conditions

The teneral energy content of field-derived flies was more variable than that of the selection line flies, as was anticipated. However, all energy components again increased with body size or showed a marginal trend (Table 3; Fig. 4), although the lipid content of field-derived flies

Table 2. Results of nested analysis of covariance (ANCOVA) of lipid, glycogen, and glucose content of teneral line flies of generation 11 with selection line, sex, and dung treatment combinations nested in two replicates and body size as the covariate (the three-way interaction was non-significant, so it was dropped, shifting its degrees of freedom into the replicate term), plus the analogous factorial ANCOVA at generation 24

		Lipids			Glycogen			Glucose		
	d.f.	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Generation 11										
Intercept	1	0.021	0.962	0.327	0.021	2.176	0.141	0.063	6.769	0.010
Line	2	0.150	1.209	0.328	0.198	2.750	0.098	0.024	0.774	0.480
Sex	1	1.311	10.544	**0.006**	0.082	1.141	0.304	0.036	1.156	0.300
Dung	1	3.418	27.496	**<0.001**	0.014	0.200	0.662	0.001	0.019	0.893
Body size	1	3.100	24.938	**<0.001**	0.735	10.188	**0.007**	0.917	29.647	**<0.001**
Line × sex	2	0.016	0.132	0.878	0.002	0.031	0.970	0.006	0.205	0.817
Line × dung	2	0.467	3.760	**0.049**	0.011	0.157	0.856	0.052	1.686	0.221
Sex × dung	1	1.914	15.393	**0.002**	0.136	1.892	0.191	0.033	1.067	0.319
Replicates (line × sex × dung)	14	0.124	4.596	**<0.001**	0.072	7.521	**<0.001**	0.031	3.293	**<0.001**
Error	488	0.022			0.010			0.001		
Generation 24										
Intercept	1	0.129	10.250	0.002	0.038	5.365	0.022	0.014	1.389	0.241
Line	2	0.138	11.012	**<0.001**	0.029	4.061	**0.020**	0.099	10.043	**<0.001**
Sex	1	0.301	23.970	**<0.001**	0.006	0.918	0.340	0.024	2.386	0.125
Dung	1	0.005	0.383	0.537	0.181	25.671	**<0.001**	0.051	5.198	**0.025**
Body size	1	2.422	193.006	**<0.001**	0.346	48.980	**<0.001**	0.313	31.711	**<0.001**
Line × sex	2	0.053	4.198	**0.017**	0.041	5.753	**0.004**	0.033	3.303	**0.040**
Line × dung	2	0.056	4.475	**0.013**	0.031	4.360	**0.015**	0.005	0.504	0.605
Sex × dung	1	0.049	3.881	**0.051**	0.001	0.102	0.750	0.008	0.772	0.381
Line × sex × dung	2	0.054	4.273	**0.016**	0.018	2.542	0.083	0.020	2.061	0.132
Error	112	0.013			0.007			0.010		

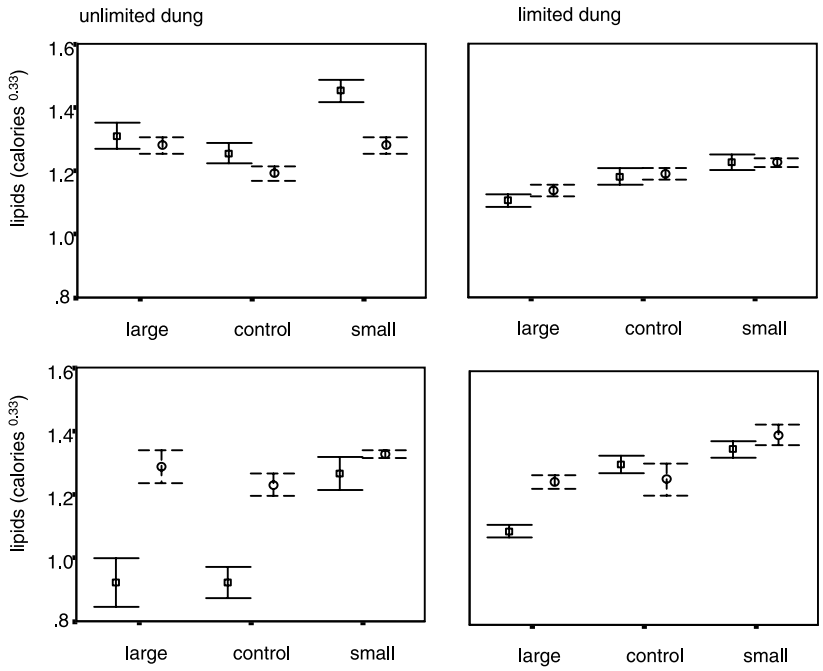

Fig. 5. Mean size-corrected lipid content (± standard error) of male and female body size selection line yellow dung flies of the 11th (top) and the 24th generation (bottom) under different dung conditions (both replicates combined). □, male; ○, female.

increased less steeply with body size than in line flies (differences in slopes: $t_{587} = 2.22$, $P < 0.05$; the slopes for glucose and glycogen were not different: $P > 0.2$; Fig. 4). Lipid and glycogen stores were influenced by rearing temperature: while glycogen content was higher at 20°C, significantly more lipids accumulated at the stressfully high 25°C (Fig. 7). Furthermore, for both energy components males accumulated more energy at the low temperature and females more at the high temperature (temperature × sex interactions in Table 3; Fig. 7), which for lipids, however, was only true under unlimited dung conditions, resulting in a three-way temperature × sex × dung interaction (Table 3, Fig. 7).

DISCUSSION

Our results confirm that body size has a strong impact on energy content and hence resistance to starvation. As in vertebrates (Peters, 1983; Calder, 1984) and some other insects (Partridge and Farquhar, 1983; Møller *et al.*, 1989; Burpee and Sakaluk, 1993; Briegel *et al.*, 2001), large teneral (i.e. freshly emerged) yellow dung flies live longer than small individuals (in absolute terms) based on their energy content (Fig. 1), although in some other insects life span was found to be independent of body size (Stockhoff, 1991; Nilssen, 1997; Strohm and Lechner, 2000). Here, we could (indirectly) relate the longer life span of large flies under complete starvation (i.e. starvation resistance) to their absolutely greater energy content at eclosion (cf. Pullin, 1987; Hoffmann *et al.*, 2001). Our finding that large individuals have greater starvation resistance (Fig. 1) is consistent with the physiological scaling relationships expected (Calder, 1984; Lindstet and Boyce, 1985) and partly confirmed here: while storage tissues tend to scale isometrically with body size (found here for lipids but not for

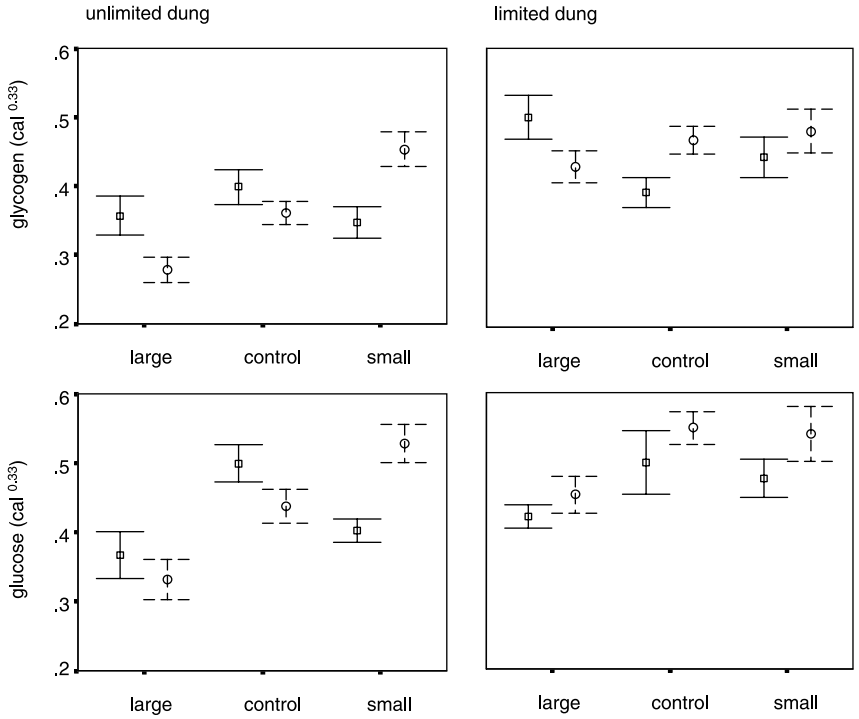

Fig. 6. Mean size-corrected glycogen (± standard error; top) and glucose content (± standard error; bottom) of female and male body size selection line yellow dung flies of generation 24 under unlimited (left) and limited (right) dung conditions. □, male; ○, female.

glycogen and glucose: Fig. 4), energy expenditure is expected to scale hypo-allometrically with size, following Kleiber's (1932) 3/4-power law. Thus larger individuals have surplus energy that can be invested in survival, clear evidence for the net positive effect of the relative efficiency hypothesis. Interestingly, however, and opposing this finding, we found that teneral individuals of our small body size selection lines lived relatively (i.e. when corrected for body size) longer (Fig. 2) and started with relatively more energy (Figs. 5 and 6) than flies selected for large size (which did not differ from the controls). By accumulating relatively more energy during the larval growth period, presumably at least partly mediating their greater starvation resistance, small line flies thus showed a correlated (genetic) response to body size selection, implying a partial compensation of their disadvantage of being small. In this context, we also note the albeit slightly but consistently steeper relationship between energy content and body size for our lines compared with field-derived flies (Fig. 4), presumably reflecting this correlated evolution to body size selection and/or some sort of adaptation to the laboratory environment. Similar reductions in pre-adult viability (Partridge and Fowler, 1993; Santos *et al.*, 1994; Zwaan *et al.*, 1995) and increases in starvation resistance and energy content (Harshman *et al.*, 1999) associated with larger body size have been found in studies of *Drosophila*, although overall the latter relationships are inconsistent (e.g. Robinson *et al.*, 2000; Hallas *et al.*, 2002).

To gain more energy in the pre-adult (larval or pupal) stage resulting in higher teneral energy reserves, several mechanisms are conceivable (Kause *et al.*, 1999). One is that larvae of the

Table 3. Results of analysis of covariance of lipid, glycogen, and glucose reserves of teneral field-derived flies with sex, pre-adult temperature, and dung treatment as fixed factors and body size as the covariate

		Lipids			Glycogen			Glucose		
	d.f.	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Intercept	1	0.000	0.001	0.981	0.000	0.057	0.812	0.005	1.293	0.258
Pre-adult temperature	1	0.903	20.168	**<0.001**	0.040	5.702	0.019	0.010	2.475	0.119
Dung	1	0.124	2.758	0.100	0.000	0.037	0.848	0.000	0.123	0.727
Sex	1	0.080	1.787	0.184	0.003	0.431	0.513	0.009	2.200	0.141
Body size	1	0.186	4.142	**0.044**	0.025	3.539	0.063	0.053	13.368	**<0.001**
Pre-adult temperature × dung	1	0.001	0.012	0.913	0.002	0.336	0.563	0.007	1.799	0.183
Pre-adult temperature × sex	1	0.306	6.821	**0.010**	0.029	4.200	**0.043**	0.008	2.078	0.152
Dung × sex	1	0.168	3.746	0.056	0.019	2.659	0.106	0.001	0.148	0.701
Pre-adult temperature × dung × sex	1	0.371	8.277	**0.005**	0.010	1.500	0.224	0.003	0.707	0.402
Error	100	0.045			0.007			0.004		

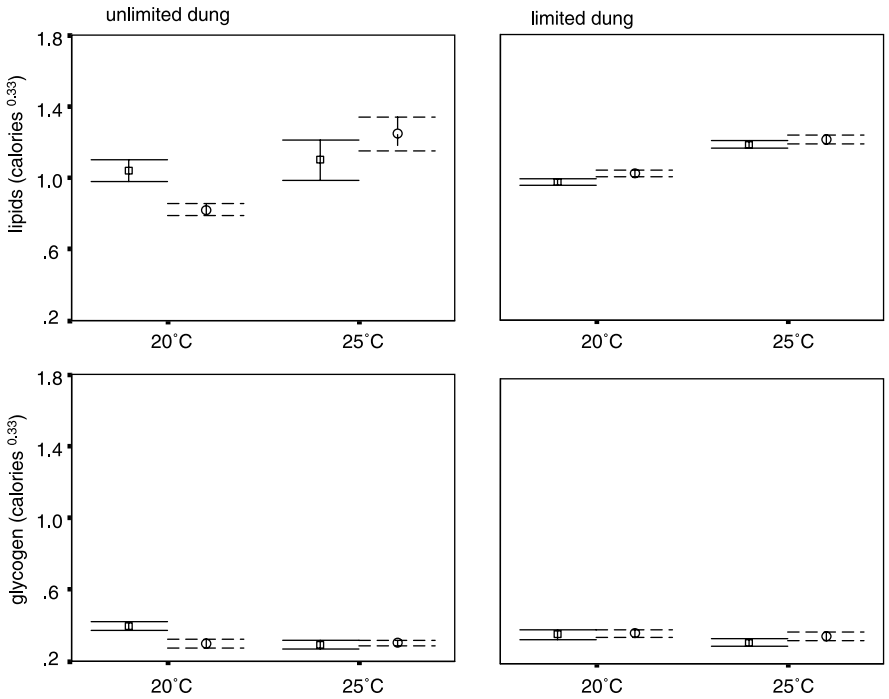

Fig. 7. Mean size-corrected lipid (± standard error) and glycogen content (± standard error) of male and female field-derived yellow dung flies at different dung and larval temperature conditions. □, male; ○, female.

small line can eat more food per unit time. Alternatively, small larvae may better absorb and assimilate food or store more energy (Scriber and Slansky, 1981; Schroeder and Lawson, 1992; Joshi and Mueller, 1996; Bochdanovits and de Jong, 2003). Additionally, small individuals may not need to invest as much energy in maintenance and growth, which they can then allocate to energy stores. A further mechanism may be a (correlated) change in metabolic rate. In the literature, there are multiple examples of changing metabolic rates due to temperature or starvation (Hack, 1997; Harshman *et al.*, 1999; Chappell and Rogowitz, 2000; Rogowitz and Chappell, 2000; Gillooly *et al.*, 2001; Fielden *et al.*, 2004). If metabolic rate decreased in the small line, then small individuals would need less energy for the same physiological processes and could store the excess energy. This last potential mechanism directly relates to our initially proposed scaling hypotheses: large animals have higher energy content and a relatively more efficient energy use than small individuals. However, in absolute terms they need more energy to sustain their body functions. Consequently, the relationships between energy content and body size on the one hand (presumed isometric; cf. above) and energy consumption and body size on the other (Kleiber's law: hypo-allometric) changed differently in the three selection lines such that small line flies were able to store relatively more energy than large line individuals. However, as we only ever see the net effects, these scaling relationships are difficult to compare, so the underlying physiological mechanisms will need to be investigated more directly in the future.

Typically in yellow dung flies, limited dung – which is common in nature due to inter- and intra-specific competition as well as variable dung pat sizes – strongly reduces phenotypic

body size (Amano, 1983; Blanckenhorn, 1998). As might be expected, we found that abundant dung (i.e. larval food) increases adult starvation resistance, at least in part due to higher energy reserves of adults emerging from unlimited dung. Nevertheless, we also found that relative to body size energy content was greater at limited dung, and survival was not reduced at an adult temperature of 25°C (whereas it was at 20°C; Fig. 3). This makes sense when limited larval conditions predict poor adult conditions (e.g. Gage, 1995), although in our species this is unlikely to be the case because yellow dung fly adults are predatory. Nevertheless, relatively greater teneral energy reserves can smooth the start into any unfavourable adult environment. However, the compensatory effect of pre-adult food conditions on energy content and life span was small relative to that of body size *per se*, and its effect on early adult reproduction was also small but not negligible (Reim *et al.*, in press).

Temperature is also known to influence body size, energy content, and longevity: high temperature during larval development generally leads to smaller adult body size in insects – the temperature–size rule (Atkinson, 1994; Partridge *et al.*, 1994; Blanckenhorn, 1997; Nunney and Cheung, 1997; Angilletta and Dunham, 2003). This was also the case in the present study. However, there is no consistent pattern of how temperature affects energy accumulation and reserves in larvae or in adults in general, as many processes such as gut passage time, ingestion, and assimilation efficiency are involved (reviewed by Scriber and Slansky, 1981; Kause *et al.*, 1999). For example, assimilation efficiency, an important factor influencing energy accumulation, seems to be independent of temperature for most insects but may increase with temperature, as shown in the eastern tent caterpillar *Malacosama americanum* (Schroeder and Lawson, 1992). In contrast, Briegel and Timmermann (2001) found decreasing lipid, glycogen, and glucose stores with increasing temperature in teneral mosquitoes. For field-derived yellow dung flies, we found increased lipid reserves when larvae were reared at the higher temperature of 25°C, while the reverse occurred for glycogen (Fig. 7). In yellow dung flies, higher lipid (but not glycogen) reserves at stressfully hot temperatures occur consistently in the field and the laboratory, and presumably increase survivorship in a reproductively quiescent state during hot summers (Blanckenhorn *et al.*, 2001). Nevertheless, high temperatures during adulthood decreased starvation resistance (Fig. 3). This is most likely due to metabolic rates generally increasing with temperature (Schmidt-Nielsen, 1997; Willmer *et al.*, 2000), so that a fixed amount of energy is used up more quickly (cf. Krasnov *et al.*, 2002; Renault *et al.*, 2003). Higher temperatures during the pre-adult stage, in contrast, did not consistently affect adult survival after eclosion in this study (although it tended to increase: Fig. 3), even though high temperatures during the pre-adult stage typically predict high temperatures for adult flies (Blanckenhorn *et al.*, 2001). Thus the beneficial effect of greater lipid reserves as a life-history response to high temperatures remains unclear in yellow dung flies (Blanckenhorn *et al.*, 2001).

We found greater survival propensity of females based on greater energy stores when correcting for the body size difference between the sexes (Fig. 3). However, that the average life span of females is longer than that of males is rather a common phenomenon in animals (Hazzard, 1986; Smith and Warner, 1989) and is also true for several insect species [e.g. parasitic wasps (Costamagna and Landis, 2004); fleas (Krasnov *et al.*, 2002); eucalyptus-boring beetles (Rogowitz and Chappell, 2000)], but has not been reported for yellow dung flies before. Yet, Nespolo *et al.* (2003) considered longer female survival only to be a side-effect of the generally larger body size found in female insects. In contrast, Fox *et al.* (2003) compared the life spans of two seed beetles with male-biased (*Stator limbatus*) and female-biased (*Callosobruchus maculatus*) size dimorphism: in both species females outlived males. Our results are in line with those of Fox *et al.* (2003), as yellow dung fly females are smaller than males but nevertheless lived longer

(when corrected for body size). We do not interpret this effect as a correlated response to the selection regime. Rather, the greater life span and larger energy accumulation of females likely result from the fundamentally different life histories of the sexes, as the field-derived flies also showed differences in energy accumulation between the sexes, although this depended somewhat on rearing temperature (Fig. 7). A consistent pattern here was that generally the larger males suffered disproportionately more under multiple stresses, which has been noted before and could relate directly to their larger size (Blanckenhorn, 1997, 1998; Hosken *et al.*, 2000).

The evidence presented here reveals a complex picture of energetics and its relationship to initial adult survival after eclosion in yellow dung flies. Many extrinsic ecological factors, most notably stressful temperatures and larval food limitation, as well as intrinsic factors such as body size and sex affect survival, starvation resistance, and energy accumulation in complex ways. With regard to our initial question about the relative importance of the absolute energy demand hypothesis versus the relative efficiency hypothesis, we conclude that under most conditions, the greater metabolic efficiency of large-sized individuals more than compensates for the disadvantages of their higher absolute energy demands, and thus has to be considered more important for early adult survival. This is in line with scaling relationships typically showing that energy stores scale mostly isometrically, while energy expenditure scales hypo-allometrically with body size (Kleiber, 1932; Calder, 1984). Nevertheless and importantly, we also showed that in a correlated response to artificial selection on body size, small individuals improved relative to large individuals in terms of energy accumulation and starvation resistance. We are aware that some of our selection line investigations are unreplicated due to crossing the replicate lines. However, at minimum we investigated a genetic body size gradient with three points (small, control, large) as well phenotypic gradients (due to food limitation and temperature). Clearly, however, we found no evidence whatsoever for energetic hypotheses causing viability selection against large body size (Blanckenhorn, 2000, 2005).

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